Environmental Toxicology and Chemistry, Vol. 22, No. 12, pp. 3056–3061, 2003

Printed in the USA
0730-7268/03 $12.00 + .00

AGRICULTURAL ADJUVANTS: ACUTE MORTALITY AND EFFECTS ON POPULATION GROWTH RATE OF *DAPHNIA PULEX* AFTER CHRONIC EXPOSURE

JOHN D. STARK*† and WILLIAM K. WALTHALL‡
†Washington State University, Puyallup Research and Extension Center, Puyallup, Washington 98371, USA
‡URS Corporation, 1400 Century Square, 1501 4th Avenue, Seattle, Washington 98101, USA

(*Received* 23 *October* 2002; *Accepted* 13 *May* 2003)

Abstract—Acute and chronic toxicity of eight agricultural adjuvants (Bond®, Kinetic®, Plyac®, R-11®, Silwet L-77®, Sylgard 309®, X-77®, and WaterMaxx®) to *Daphnia pulex* were evaluated with 48-h acute lethal concentration estimates (LC50) and a 10-d population growth-rate measurement, the instantaneous rate of increase (r_i). Based on LC50, the order of toxicity was R-11 > X-77 = Sylgard 309 = Silwet L-77 > Kinetic > Bond > Plyac > WaterMaxx; all LC50 estimates were higher than the expected environmental concentration (EEC) of 0.79 mg/L, indicating that none of these adjuvants should cause high levels of mortality in wild *D. pulex* populations. Extinction, defined as negative population growth rate, occurred after exposure to 0.9 mg/L R-11, 13 mg/L X-77, 25 mg/L Kinetic, 28 mg/L Silwet, 18 mg/L Sylgard, 450 mg/L Bond, 610 mg/L Plyac, and 1,600 mg/L WaterMaxx. Concentrations that caused extinction were substantially below the acute LC50 for R-11, Kinetic, Plyac, X-77, and Bond. The no-observable-effects concentration (NOEC) and lowest-observable-effects concentration (LOEC) for the number of offspring per surviving female after exposure to R-11 were 0.5 and 0.75 mg/L, respectively. The NOEC and LOEC for population size after exposure to R-11 were 0.25 and 0.5 mg/L, respectively. Both of these values were lower than the EEC, indicating that R-11 does have the potential to cause damage to *D. pulex* populations after application at recommended field rates. The wide range of concentrations causing extinction makes it difficult to generalize about the potential impacts that agricultural adjuvants might have on aquatic ecosystems. Therefore, additional studies that examine effects on other nontarget organisms and determine residues in aquatic ecosystems may be warranted.

Keywords—Adjuvants Toxicity *Daphnia* Population growth rate

INTRODUCTION

Agricultural adjuvants are substances that, once added to a pesticide spray tank, modify a pesticide's performance and the physical properties of the spray mixture. Adjuvants are often added to pesticides prior to application [1], and they are designed to act as wetting agents, spreaders [2], stickers, emulsifiers, dispersing agents, drift-control agents [3], foam suppressors, and penetrants.

Some agricultural adjuvants increase pesticide efficacy [4] and modify environmental fate [5]. Results of previous studies have indicated that some of these products are toxic to certain species on their own or they increase the toxicity of pesticides [4,6–11]. However, these products are not regulated under the Federal Insecticide, Fungicide, Rodenticide Act (1996) when used as additives to registered pesticides; thus, toxicity data are not required by the U.S. Environmental Protection Agency [12].

A large body of literature exists concerning the toxicity of nonagricultural surfactants and, in particular, nonylphenol and linear alkylbenzene sulfonate, to various species [13–19]. Although some agricultural adjuvants contain linear alkylbenzene sulfonate, many do not. Because these agricultural adjuvants are being applied to agricultural ecosystems, along with pesticides, there is the possibility for these products to enter freshwater ecosystems through direct application, runoff, and atmospheric deposition; however, the potential effects on aquatic organisms following these potential routes of exposure have not been thoroughly studied.

* To whom correspondence may be addressed (stark@puyallup.wsu.edu).

The objective of this study was to determine the toxicity of the agricultural adjuvants Bond®, Kinetic®, Plyac®, R-11®, Silwet L-77®, Sylgard 309®, X-77®, and Watermaxx® to the cladoceran, *Daphnia pulex* (Leydig). The acute toxicity of these adjuvants was evaluated using traditional 48-h acute lethal concentration estimates and the chronic toxicity was assessed using a 10-d measure of population growth rate, the instantaneous rate of increase [20].

MATERIALS AND METHODS

Test organisms

Daphnia pulex, obtained from cultures maintained at the Washington State University Research and Extension Center (Puyallup, WA) were reared in reconstituted dilution water (RDW) inside a freestanding environmental chamber set at 25 ± 0.1°C, 50% RH, and a 16:8-h light:dark regimen. All RDW used in this study was prepared according to a method modified from a U.S. Environmental Protection Agency protocol [21] resulting in a RDW with pH 7.4 to 7.8, conductivity 260 to 320 μS, dissolved oxygen >8.0 mg/L, and alkalinity of 60 to 70 mg/L, and a hardness of 80 to 100 mg/L. This synthetic freshwater corresponds to a classification of moderately hard.

Daphnia cultures were renewed daily and fed 0.3 ml feeding solution. The feeding solution contained a 1:1 mixture of yeast-cereal leaves-trout chow and the algal species *Selenastrum capricornutum* that was originally purchased from Charles River (Wilmington, MA, USA).

Adjuvants evaluated

The eight agricultural adjuvants evaluated for this study were Bond, Kinetic, Plyac, R-11, Silwet L-77, Sylgard 309, WaterMaxx, and X-77. The adjuvants R-11 and X-77 are nonionic surfactants. The R-11 contains octyl phenoxy polyethoxy ethanol/butyl alcohol (octylphenol), a known endocrine disruptor; Silwet L-77 and Sylgard 309 are organosilicone surfactants. Kinetic is a nonionic-organosilcone blend; Bond and Plyac are spreader/stickers. Furthermore, the principal functioning agents of Plyac are emulsifiable oxidized polyethylene and ethoxylated phenoxy alcohol; and WaterMaxx is a blended nonionic soil penetrant/wetting agent with nonionic polyol as the principal functioning agent.

Both R-11 and Sylgard 309 are distributed by Wilbur-Ellis (Fresno, CA, USA) and Bond, Plyac, Silwet L-77, and X-77 are distributed by Loveland (Greeley, CO, USA). Kinetic is distributed by Helena Chemical (Memphis, TN, USA) and WaterMaxx, is distributed by Western Farm Services (Fresno, CA, USA). Some selected physical and chemical properties of these adjuvants are presented in Table 1.

Acute toxicity

Adjuvant concentrations were prepared by serial dilution from newly prepared stock solutions in 100 ml RDW. The range of adjuvant concentrations causing an effect on *D. pulex* was initially determined with 10-fold serial dilutions. Thereafter, 5 to 8 concentrations causing 10 to 90% mortality were examined for each adjuvant. Five third filial generation (F_3) neonates (<24 h old) were transferred into 30-ml plastic cups containing 25 ml of sample solution for each concentration tested. *Daphnia* were fed 2 h before introduction to the test solutions. Test organisms were kept in environmental chambers set at the same conditions listed above for rearing. The test was static, nonrenewable, and *D. pulex* mortality was assessed at 48 h. *Daphnia* were considered dead when there was no movement of the external and thoracic appendages or the heart following gentle prodding with a glass pipette following observation under microscopic magnification. This experiment was replicated five to six times on different days with different generations of *D. pulex*.

Population growth-rate studies

Effects of the adjuvants on *D. pulex* population growth rate were determined using a measure called the instantaneous rate of increase (r_i) (Eqn. 1) [22–28]. The instantaneous rate of growth is a direct measure of population increase and is calculated by the following equation:

$$r_i = \ln(N_f/N_o)/t \quad (1)$$

where N_f is the final number of animals, N_o is the initial number of animals, and t is the change in time (number of days the experiment was run). Solving for r_i yields a rate of population increase or decline similar to that obtained by the intrinsic rate of increase (r_m) [20]. Positive values of r_i indicate a growing population, $r_i = 0$ indicates a stable population, while a negative r_i value indicates a population in decline and headed toward extinction.

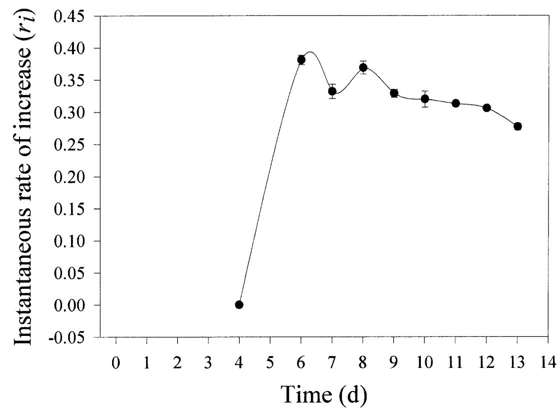

Fig. 1. Instantaneous rate of increase (r_i) versus time for *Daphnia pulex* control population. Bars around each mean are standard errors.

Logarithmically spaced concentrations (5–8) and a control (RDW) were prepared by serial dilution from a fresh stock solution. For each concentration, 10 neonates (<24 h old) were added to 100 ml of test solution in 296-ml SOLO® Ultra Clear™ plastic cups (Chicago, IL, USA) using a small-bore disposable glass pipette. Following introduction of *D. pulex* to the cups, 1.0 ml of feeding solution, prepared as previously described, was added to each cup. One milliliter of the feeding solution described above was added to each cup daily. After 10 d of exposure, all cups were removed from the environmental chamber and the number of original adults and their offspring were counted. Day 10 was chosen as the census date because it provided enough time for control populations introduced as neonates to produce multiple broods of progeny [28]. Furthermore, a plot of r_i for the control over time indicated that growth rates became stable by day 9 and that running the experiment longer than 10 days provided little additional information (Fig. 1).

Daphnia were considered dead when there was no movement of the external and thoracic appendages or the heart following gentle prodding with a glass pipette following observation under microscopic magnification. The instantaneous

Table 1. Selected physical and chemical properties of the adjuvants. NE = not estimated

Chemical	Boiling point (°C)	Vapor pressure	Specific gravity (g/ml)	Water solubility
Bond®[a]	100	NE	1.01	Soluble
Kinetic®[b]	>150	<1 mm HG	1.03	Dispersible
Plyac®[a]	NE	NE	0.998	Emulsifies
R-11®[c]	NE	NE	1.02	Slightly soluble (10%)
Silwet L-77®[a]	100	<1 mm HG	1.01	Dispersible
Sylgard 309®[c]	NE	NE	1.03	Soluble
Water Maxx®[d]	43	NE	1.02	Soluble
X-77®[a]	80	NE	0.98	Soluble

[a] Distributed by Loveland (Greeley, CO, USA).
[b] Distributed by Helena (Memphis, TN, USA).
[c] Distributed by Wilbur-Ellis (Fresno, CA, USA).
[d] Distributed by Western Farm Services (Fresno, CA, USA).

Table 2. Acute toxicity of eight agricultural adjuvants to the aquatic cladoceran *Daphnia pulex* following 48-h exposure[a]

Adjuvant	Slope (± standard error)	LC50[b] (95% confidence limit) (mg/L)[c]	Ratio LC50/slope
Bond®	7.64 (± 1.56)	614 (558–706)	80
Kinetic®	5.32 (± 0.63)	111 (99.6–121)	21
Plyac®	2.82 (± 0.52)	2,666 (1,418–4,155)	945
R-11®	6.55 (± 1.11)	13.2 (12.5–14.0)	2
Silwet L-77®	6.21 (± 1.38)	23.4 (17.8–28.9)	4
Sylgard 309®	2.89 (± 0.64)	22.9 (17.9–27.2)	8
Water Maxx®	3.46 (± 0.70)	16,334 (13,607–19,322)	4,721
X-77®	10.90 (± 2.43)	16.4 (15.0–19.2)	2

[a] Chemical manufacturers are listed in Table 1.
[b] Lethal concentration that causes 50% mortality.
[c] Number of individuals tested for each adjuvant ranged from 150 to 270.

r_i for each population was calculated using Equation 1. This experiment was replicated a minimum of three times for each adjuvant. The number of offspring per surviving female for the most toxic compound, R-11, was also determined.

Comparison of toxicity endpoints to the expected environmental concentration

The expected environmental concentration (EEC) is defined as the concentration of pesticide in 15 cm of water after a direct overspray of a forest at the maximum application rate [29]. The recommended application rate of the agricultural adjuvants evaluated in this study is 1,182 g/ha (1 U.S. pint/acre). Following the procedure outlined by the Canadian Pest Management Regulatory Agency (Ottawa, ON), the approximate EEC for the adjuvants tested was estimated to be 0.79 mg/L. This EEC value was compared with the toxicity endpoints developed in this study.

Statistical analysis

Acute concentration–mortality regressions were estimated by probit analysis [30] using the SAS probit procedure [31] following correction for control mortality using Abbott's formula [32]. Control mortality never exceeded 5%. Differences in toxicity were considered significant when 95% confidence limits did not overlap.

For R-11 only, data on population size (number of individuals at the end of the chronic study) and the number of offspring per surviving female were subjected to Shapiro–Wilk's test (normality) and Bartlett's test (equal variance). If the data met the requirements of both tests, the no-observable-effects concentration (NOEC) and the lowest-observable-effects concentration (LOEC) were determined using Dunnett's test (1 tail, $p = 0.05$) [33].

RESULTS

Acute toxicity

Forty-eight-hour LC50 estimates for each adjuvant are presented in Table 2. A wide range of sensitivity was exhibited by *D. pulex* to the adjuvants; R-11 was significantly more toxic than all of the other adjuvants in terms of 48-h LC50. Based on 95% confidence limit overlap, the order of toxicity was R-11 > X-77 = Sylgard 309 = Silwet L-77 > Kinetic > Bond > Plyac > WaterMaxx. All of the LC50 estimates were higher than the EEC (0.79 mg/L), indicating that none of these surfactants should cause high levels of mortality in wild *D. pulex* populations. The slopes of the probit curves differed among the adjuvants. Differences in slopes can indicate differences in modes of action. A comparison of the ratio of the LC50 to the slope indicated that there was no uniform relationship (Table 2). This is probably due to differences in the mode of action of these compounds and/or differences in uptake and/or excretion.

Population growth-rate effects

A concentration–response relationship was observed between adjuvant exposure concentration and negative population growth rate (Fig. 2). Concentrations that caused extinction (defined as negative population growth rate) of *D. pulex* populations are listed in Table 3. Based on concentrations that caused extinction, R-11 was far more toxic than the other adjuvants. The next most toxic adjuvant was X-77, followed by Sylgard 309, Kinetic, Silwet L-77, Bond, Plyac, and WaterMaxx (Table 3, Fig. 2).

Relationship between 48-h acute mortality and extinction

The corresponding 48-h acute lethal concentration estimate where r_i becomes negative is presented in Figure 2. Concentrations that caused extinction were substantially below the acute LC50 for R-11 (<LC1), Kinetic (<LC1), Plyac (LC5), X-77 (LC10), and Bond (LC15), indicating that irreversible population effects occurred after exposure to concentrations that were acutely nontoxic.

The ratio between the acute 48-h LC50 and extinction concentration is presented in Table 3. A ratio of one indicates that extinction occurs around the LC50, a ratio greater than one indicates that extinction occurs at concentrations below the LC50, and a ratio less than one indicates that extinction occurs after exposure to a concentration higher than the acute LC50. Only Silwet L-77 had a ratio lower than one, indicating that it took a concentration higher than the LC50 to cause extinction. The remaining surfactants had ratios higher than one. However, Sylgard 309, X-77, and Bond had ratios only slightly higher than one, while that ratio was higher for Kinetic, Plyac, WaterMaxx, and R-11, ranging from 4.4 to 14.7. This is interesting because it indicates that concentrations of these adjuvants below the acute LC50 can cause extinction in *D. pulex*.

A comparison of the EEC and the adjuvant concentrations that caused extinction indicated that only R-11 caused extinction (0.9 mg/L) near the EEC (0.79 mg/L). The other adjuvants caused extinction at concentrations much higher than the EEC.

The R-11 was much more toxic to *D. pulex* after chronic exposure compared with acute exposure (Fig. 3). For example,

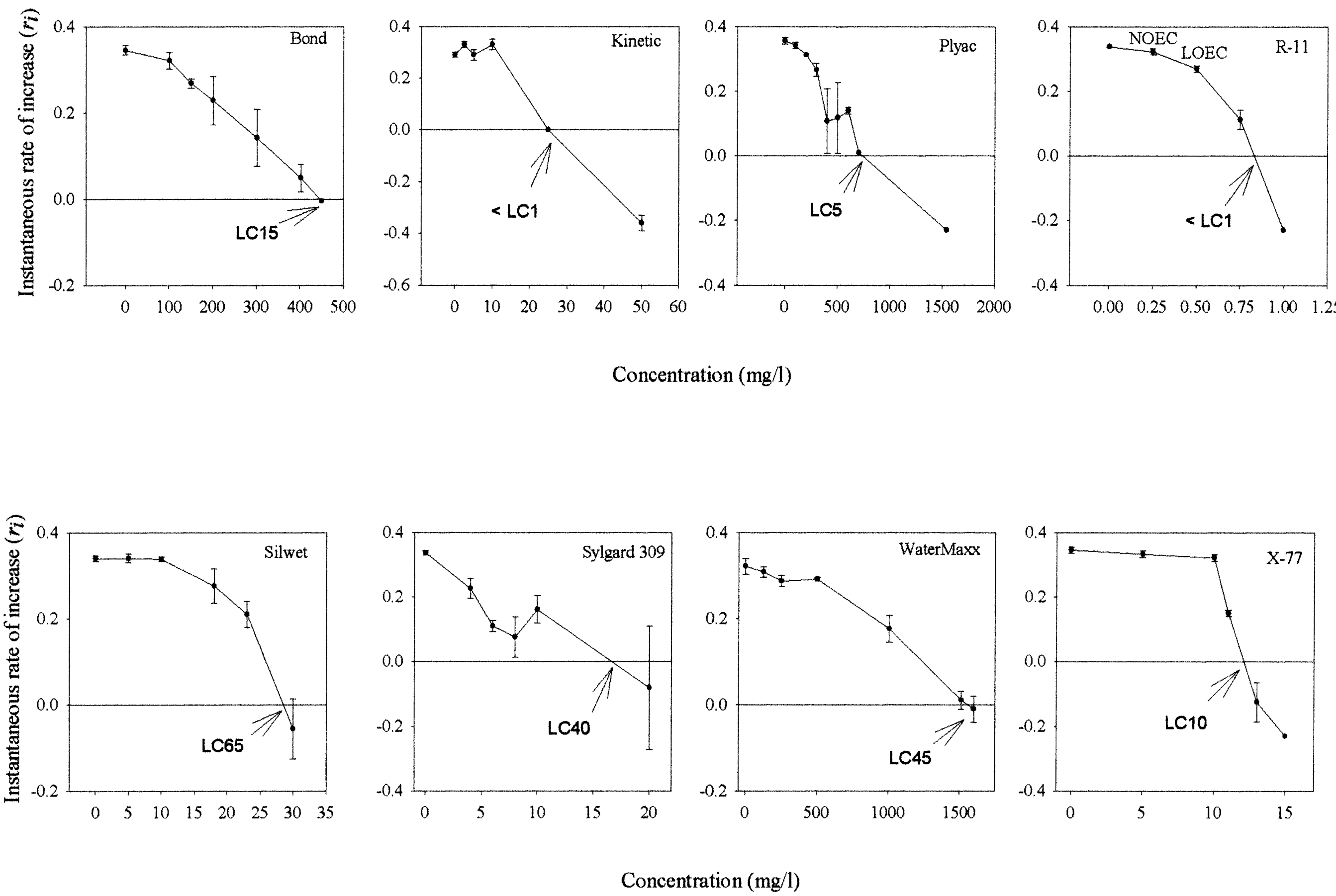

Fig. 2. Effects of adjuvants on *Daphnia pulex* instantaneous rate of increase (r_i). Bars around each mean are standard errors. Lethal concentration (LC) value is the corresponding 48-h acute lethal concentration estimate at the concentration where growth rate becomes negative (extinction). NOEC = no-observable-effect concentration; LOEC = lowest-observable-effect concentration. Chemical manufacturers are listed in Table 1.

20% mortality occurred after exposure to a concentration 20 times lower in the chronic study compared with the acute study (Fig. 3).

NOEC and LOEC for R-11

The R-11 data on the number of offspring per surviving female met the requirements of both the Shapiro–Wilk's normality test ($p > 0.01$) and Bartlett's equal variance test ($p = 0.03$). R-11 had a negative effect on *D. pulex* reproduction, as indicated by the decline in the number of offspring per surviving female (Fig. 4). The NOEC and LOEC for number of offspring per surviving female were calculated to be 0.5 and 0.75 mg/L, respectively (Fig. 4).

Table 3. Ratio of 48-h acute LC50 and extinction concentration[a]

Adjuvant	Extinction concentration (mg/L)	Ratio (acute LC50[b]/extinction concentration)
Bond	450	1.4
Kinetic	25	4.4
Plyac	610	4.4
R-11	0.9	14.7
Silwet L-77	28	0.8
Sylgard 309	18	1.3
X-77	13	1.3
Water Maxx	1,600	10.2

[a] Chemical manufacturers are listed in Table 1.
[b] Lethal concentration that causes 50% mortality.

The R-11 data on population size met the requirements of the Shapiro–Wilk's normality test ($p > 0.01$) and Bartlett's equal variance test ($p = 0.19$). The NOEC and LOEC for population size were 0.25 and 0.5 mg/L, respectively (Fig. 2).

The LOEC for reproduction and population size were both lower than the EEC of 0.79 mg/L, indicating that R-11 does have the potential to cause damage to *D. pulex* populations after application at recommended field rates.

DISCUSSION

The mode of action of silicon agricultural adjuvants, at least for insects, is thought to be suffocation [9]. For aquatic invertebrates, the mode of action has not been determined. However, most of the adjuvants we tested caused lethal and sublethal effects. For example, R-11 negatively affected *D. pulex* reproduction, indicating that a mode of action other than suffocation was occurring. Somehow, R-11 is interfering with the ability to produce offspring, but the actual mechanism by which this occurs was not tested for in this study. However, R-11 contains octylphenols, which are known endocrine disruptors. Jobling et al. [34] showed that octylphenol affects testicular growth and plasma vitellogenin induction in adult male rainbow trout and acts as a xenoestrogen.

A wide range of toxicity was observed for the eight agricultural adjuvants evaluated in this study spanning several orders of magnitude. The R-11 was the most toxic adjuvant based on both 48-h acute mortality and extinction. The order of toxicity of the adjuvants was basically the same for each

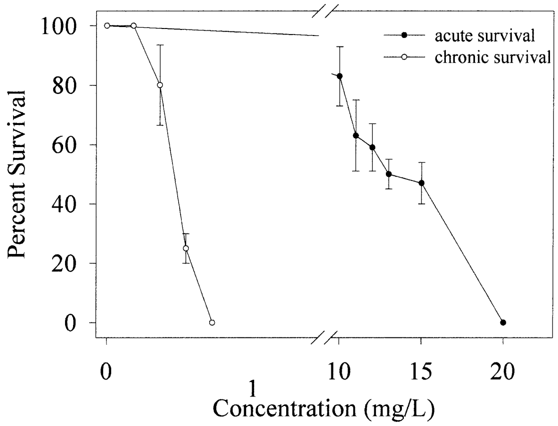

Fig. 3. Acute and chronic survival of *Daphnia pulex* after exposure to R-11® (Wilbur-Ellis, Fresno, CA, USA). Bars around each mean are standard errors.

toxicity endpoint evaluated (acute LC50 or extinction). However, it was important to evaluate toxicity at the population level because sublethal effects were occurring. Population-level measurements of toxicity combine both lethal and sublethal effects and therefore a measure of the total toxic effect on a population can be obtained [35]. Forbes and Calow [36] have found that demographic toxicological data are superior to other types of toxicity data and in fact a recent book on the use of different toxicity metrics has been published [37].

The R-11 caused extinction after exposure to 0.9 mg/L, but the LC50 for R-11 was estimated to be 13 mg/L. Thus, R-11 was almost 15 times more toxic than predicted by the acute LC50. Extinction occurred after exposure to concentrations lower than the LC50 for most of the adjuvants tested. Therefore, the LC50 did not give an accurate estimate of total toxic effect for these adjuvants.

In other studies, agricultural adjuvants were found to be toxic to nontarget aquatic organisms. Henry et al. [6] found that X-77, an adjuvant commonly applied with herbicide Rodeo® (Monsanto, St. Louis, MO, USA), was much more toxic to aquatic invertebrates than Rodeo. The laboratory-derived 48-h LC50 and 95% confidence limit (CL) for *Daphnia magna* was 2.0 (1.5–2.7) mg/L. Results of our study indicate that *D. pulex* (LC50 and 95% CL = 16.4 [15.0–19.2] mg/L) is significantly less susceptible to X-77 than *D. magna*. Mann and Bidwell [38] investigated the acute toxicity of the agricultural surfactants nonylphenol ethoxylate and alcohol alkoxylate to the tadpoles of four Australian and two exotic frogs. They found that these products produced nonspecific narcosis in all of the species tested.

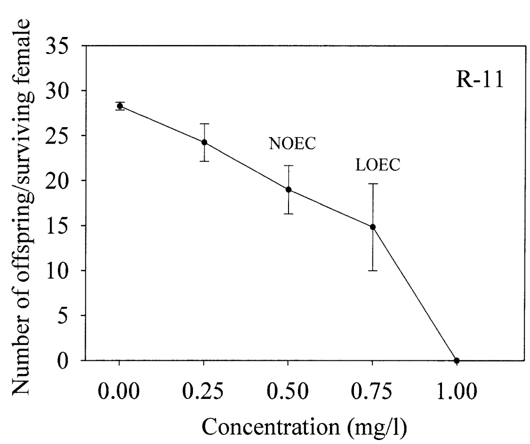

Fig. 4. Effects of R-11® (Wilbur-Ellis, Fresno, CA, USA) on the number of *Daphnia pulex* offspring per surviving female. Bars around each mean are standard errors. NOEC = no-observable-effect concentration; LOEC = lowest-observable-effect concentration.

However, contrary to the two studies mentioned above, the study by Jumel et al. [39] found that the agricultural adjuvant Agral® 90 (Imperical Chemical, London, UK; nonylphenol polyethoxylates) actually mitigated the reproductive effects of the herbicide fomesafen in the pond snail, *Lymnaea stagnalis*. Jumel et al. [39] believe that the adjuvant actually prevented fomesafen from reaching the snails in aquatic mesocosms.

Little data have been generated on the toxicity and environmental concentrations of agricultural adjuvants, and therefore it is difficult to estimate the potential hazard that these products pose to aquatic ecosystems. However, Henry et al. [6] estimated that the highest concentration of X-77 applied to wetlands as a mixture with the herbicide Rodeo would be 0.031 mg/L. This concentration of X-77 was considerably lower than the acute LC50 for *D. magna* in their study (2 mg/L) and is 387 times lower than the extinction concentration (12 mg/L) of X-77 for *D. pulex* estimated in our study. However, the persistence of X-77 in aquatic systems and its ability to bioaccumulate is not known.

In our study, we found that R-11 could be present in the environment at concentrations that could cause damage to *D. pulex* populations. Additional studies, particularly those that examine effects of agricultural adjuvants on other nontarget organisms and determine residues in aquatic systems, may be warranted based on the results obtained in this study.

REFERENCES

1. Stevens PJG. 1993. Organosilicone surfactants as adjuvants for agrochemicals. *Pestic Sci* 38:103–122.
2. Paveglio FL, Kilbride KM, Grue CE, Simenstad CA, Fresh KL. 1996. Use of Rodeo and X-77 spreader to control smooth cordgrass (*Spartina alterniflora*) in a southwestern Washington estuary. 1. Environmental fate. *Environ Toxicol Chem* 15:961–968.
3. Cai S-S, Stark JD. 1997. Evaluation of five fluorescent dyes and triethyl phosphate as atmospheric tracers of agricultural sprays. *J Environ Sci Health Part B* 32:969–983.
4. Sharma SD, Singh M. 2001. Surfactants increase toxicity of glyphosate and 2,4-D to Brazil pusley. *HortScience (Calcutta)* 36: 726–728.
5. Weinberger P, Greenhalgh R. 1984. Some adjuvant effects on the fate of fenitrothion and aminocarb. *Environ Toxicol Chem* 3:325–334.
6. Henry CJ, Higgins KF, Buhl KJ. 1994. Acute toxicity and hazard assessment of Rodeo, X-77 spreader, and Chem-Trol to aquatic invertebrates. *Arch Environ Contam Toxicol* 27:392–399.
7. Abdelghani AA, Tchounwou PB, Anderson AC, Sujono H, Heyer LR, Monkiedje A. 1997. Toxicity evaluation of single and chemical mixtures of Roundup, Garlon-3A, 2,4-D, and Syndets surfactant to channel catfish (*Ictalurus punctatus*), bluegill sunfish (*Lepomis michochirus*), and crawfish (*Procambarus* spp.). *Environ Toxicol Water Qual* 12:237–243.
8. Anastacio PM, Lutzhoft HCH, Halling-Sorensen B, Marques JC. 2000. Surfactant (Genapol OX-80) toxicity to *Selenastrum capricornutum*. *Chemosphere* 40:835–838.
9. Purcell MF, Schroeder WJ. 1996. Effects of Silwet L-77 and Diazinon on three tephritid fruit flies (Diptera: Tephritidae) and associated endoparasitoids. *J Econ Entomol* 89:1566–1570.
10. Liu TX, Stansly PA. 2000. Insecticidal activity of surfactants and oils against silverleaf whitefly (*Bemisia argentifolii*) nymphs (Homoptera: Aleyrodidae) on collards and tomato. *Pest Manag Sci* 56:861–866.
11. Mangan RL, Moreno DS. 2001. Photoactive dye insecticide formulations: Adjuvants increase toxicity to Mexican fruit fly (Diptera: Tephritidae). *J Econ Entomol* 94:150–156.

12. U.S. Code. 1947. Federal Insecticide, Fungicide, and Rodenticide Act (Federal Environmental Pesticide Control Act) 7: Title §§ 136–136y, as amended 1972, 1973, 1975, 1978, 1983, 1984, 1988, 1990, 1991 and 1996.
13. Dolan JM, Gregg BC, Cairns J Jr., Dickson KL, Hendricks AC. 1974. The acute toxicity of three new surfactant mixtures to a mayfly larvae (Isonychia). *Arch Hydrobiol* 74:123–132.
14. Lewis MA, Perry RL. 1981. Acute toxicities of equimolar and equitoxic surfactant mixtures to *Daphnia magna* and *Lepomis macrochirus*. In Branson DR, Dickson KL, eds, *Aquatic Toxicology and Hazard Assessment*, Vol 4. STP 737. American Society for Testing and Materials, Philadelphia, PA, pp 402–418.
15. Lewis MA. 1991. Chronic and sublethal toxicities of surfactants to aquatic animals: A review and risk assessment. *Water Res* 25: 101–113.
16. Comber MHI, Williams, TD, Stewart KM. 1993. The effects of nonylphenol on *Daphnia magna*. *Water Res* 27:273–276.
17. Dorn PB, Salanitro JP, Evans SH, Kravetz L. 1993. Assessing the aquatic hazard of some branched and linear nonionic surfactants by biodegradation and toxicity. *Environ Toxicol Chem* 12:1751–1762.
18. Baldwin WS, Graham SE, Shea D, Leblanc GA. 1997. Metabolic androgenization of female *Daphnia magna* by the xenoestrogen 4-nonylphenol. *Environ Toxicol Chem* 16:1905–1911.
19. DelValls TA, Forja JM, Gomez PA. 2002. Seasonality of contamination, toxicity, and quality values in sediments from littoral ecosystems in the Gulf of Cadiz (SW Spain). *Chemosphere* 46: 1033–1043.
20. Walthall WK, Stark JD. 1997. Comparison of two population-level ecotoxicological endpoints: The intrinsic (r_m) and instantaneous (r_i) rates of increase. *Environ Toxicol Chem* 16:1068–1073.
21. U.S. Environmental Protection Agency. 1991. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. EPA/600/4-90/027. Environmental Monitoring and Support Laboratory, Cincinnati, OH.
22. Birch LC. 1948. The intrinsic rate of natural increase of an insect population. *J Anim Ecol* 17:15–26.
23. Stark JD, Tanigoshi L, Bounfour M, Antonelli A. 1997. Reproductive potential: Its influence on the susceptibility of a species to pesticides. *Ecotoxicol Environ Saf* 37:273–279.
24. Banken JAO, Stark JD. 1998. Multiple routes of pesticide exposure and the risk of pesticides to biological controls: A study of neem and the seven-spot lady beetle, *Coccinella septempunctata* L. *J Econ Entomol* 91:1–6.
25. Sibly RM. 1999. Efficient experimental designs for studying stress and population density in animal populations. *Ecol Appl* 9:496–503.
26. Stark JD, Banken JAO. 1999. Importance of population structure at the time of toxicant exposure. *Ecotoxicol Environ Saf* 42:282–287.
27. Stark JD, Banks JE. 2001. Selective pesticides: Are they less hazardous to the Environment? *BioScience* 51:980–982.27.
28. Walthall WK, Stark JD. 1999. The acute and chronic toxicity of two xanthene dyes, fluorescein sodium salt and Phloxine B, to *Daphnia pulex* (Leydig). *Environ Pollut* 104:207–215.
29. Canadian Pest Management Regulatory Agency. 1993. Registration guidelines for microbial pest control agents. Bulletin Pro 93-04. Ottawa, ON.
30. Finney DJ. 1971. *Probit Analysis,* 2nd ed. Cambridge University Press, Cambridge, UK.
31. SAS Institute. 1985. *SAS® User's Guide: Statistics.* Cary, NC, USA.
32. Abbott WS. 1925. A method of computing the effectiveness of an insecticide. *J Econ Entomol* 18:265–267.
33. Tidepool Scientific Software. 1996. *TOXCALC, for Microsoft Excel*, Ver 5.0. McKinleyville, CA, USA.
34. Jobling S, Sheahan D, Osborne JA, Matthiessen P, Sumpter JP. 1996. Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. *Environ Toxicol Chem* 15:194–202.
35. Stark JD, Banks JE. 2003. Population-level effects of pesticides and other toxicants on arthropods. *Annu Rev Entomol* 48:505–519.
36. Forbes VE, Calow P. 1999. Is the per capita rate of increase a good measure of population-level effects in ecotoxicology? *Environ Toxicol Chem* 18:1544–1556.
37. Kammenga J, Laskowski R. 2000. *Demography in Ecotoxicology*. John Wiley, Chichester, UK.
38. Mann RM, Bidwell JR. 2001. The acute toxicity of agricultural surfactants to the tadpoles of four Australian and two exotic frogs. *Environ Pollut* 114:195–205.
39. Jumel A, Coutellec M-A, Cravedi J-P, Lagadic L. 2002. Nonylphenol polyethoxylate adjuvant mitigates the reproductive toxicity of fomesafen on the freshwater snail *Lymnaea stagnalis* in outdoor experimental ponds. *Environ Toxicol Chem* 21:1876–1888.